Nonparametric longitudinal methods on clinical assessment of a single intra-articular administration of IFNγ-primed allogeneic adipose stem cells (ASCs-γ) in an equine groove model of osteoarthritis

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Introduction

Osteoarthritis (OA) is a degenerative joint disease often resulting from repetitive trauma, which affects young sportive people and horses. Pre-activation (priming) of allogeneic ASCs with interferon-gamma (ASCs-γ) have been described *in vitro* to enhance immunosuppressive function and chondroprotective effects on equine cartilage explants induced with OA when compared to non-activated cells [1]. The aim of this study was to assess the clinical effects the ASCs-γ, using an equine modified groove model, reported to cause more cartilage degeneration than inflammation [2,3].

Methods

The OA induction was performed in one metacarpophalangeal joint of 16 French Standardbred horses. The experimental protocol was approved by the local ethics committee (RECH-ETIC-P003-E01). Animals were randomly divided in two groups: the control joints (CJ, n=10) didn't received any treatment and the treatment joints (TJ, n=6) received an intra-articular injection of 15 million ASCs-γ, 7 days after OA induction. ASCs were isolated, cultured, primed and characterized as previously described *in vitro* [1]. Horses respected an exercise protocol and were evaluated using an inertial sensor device. Lameness on the OA limb was considered as the total head asymmetry, vector sum (VS), above 8.5mm. Radiographic images were assessed according to Maninchedda et al. [2]. Synovial fluid analysis was performed by measuring total proteins (TP) and prostaglandin E2 (PGE2). Other biomarkers and their corresponding analysis are out of the scope of this abstract.

Data analysis was performed using R version 3.4.4 [4]. Due to the semi-quantitative nature of some dependent variables (radiographic scores and number of lame horses) and due to the violation of the homogeneity of variances (biochemical values), rank-based nonparametric methods for longitudinal data in factorial experiments were used. Null hypotheses were formulated in terms of marginal distribution functions: 1) mean distribution over time for CJ is equal to mean distribution over time for TJ, i.e., no main effect of treatment; 2) mean distribution over treatment groups for each observational day is the same, i.e., no main effect of time; 3) for each treatment t and for each observational day d, the related distribution is equal to mean distribution over time for treatment group t minus the mean distribution over treatment groups for observational day d plus the overall mean distribution, i.e., no interaction effect between treatment and time. Point estimators of the marginal distribution functions, the so-called relative treatment/marginal effects (mean of the ranks in the marginal sample divided by the total number of observations), their confidence intervals and the robust nonparametric ANOVA-type statistics [5] denoted by ATS, all available in the library nparLD [6], were used.

Results

As suggested by the increase in medians (Table 1), there was significant increase in total radiographic scores between day 0 and day 75 (ATS(df=1)=81.2, p-value<0.000) for both groups. This is explained by the significant time effect in synovial effusion (ATS(df=1)=168.5, p-value<0.000) and ostophyte formation (ATS(df=1)=34.2, p-value<0.000).

The number of lame horses had a significant interaction effect between treatment and time (ATS(df=1.82)=3.47, p-value = 0.035). As shown in Figure 1, the relative effects of treatment are not similar over time, while there's a similar increase in both groups between day 0 and day 45, relative treatment effect decline afterwards in TJ but increases in CJ. This evidence is confirmed by post-hoc analysis between day 45 and 75 (ATS(df=1)=7.23, p-value = 0.007). In the end of the study there were 8/10 lame horses in the CL group and 1/6 lame horses in the TJ group.

The ASCs-γ treatment effects in synovial fluid were assessed after the intra-articular injection. Regarding TP (Figure 2A) and PGE2 (Figure 2B) concentrations there was significant time effect (TP: ATS(df=1.34)=9.83, p-value<0.000; PGE2: ATS(df=1.53)=5.48, p-value = 0.009) but significant treatment or interaction effects were not observed.

Discussion and conclusions

In the present study, the radiographic findings show the effects of the OA groove model, by significantly increase in synovial effusion and osteophyte formation 75 days after disease induction in both groups. These findings are similar to another OA-experimental model in ponies using allogeneic primed-MSCs [7] where radiographic changes were significantly increased by 2 months and no differences between groups (controls, primed and non-primed MSCs) were found.

The OA groove model was also effective inducing lameness, as head asymmetry was increased by day 45 in both groups. The ASCs-γ effect was observed between day 45 and day 75, when the treatment group presented significantly less lame horses compared to the control group. Since all horses were subjected to exercise during the 60 days, the reduced lameness (pain) is of particular interest. Synovial fluid TP and PGE2 analysis were performed in order to evaluate the inflammatory environment inside the joint after ASCs-γ injection. Although no significant differences were found, a delayed treatment effect is also suggested by PGE2 reduction between day 45 and day 75.

This study suggests an association between the injection of 15 million ASCs-γ and consistent lameness reduction 68 days after administration. Neither radiographic scores nor synovial fluid values revealed significant interactions effects between treatment and time, suggesting that the effects of treatment are similar over time. Nevertheless, due to the limited power of this study further studies with larger samples should be conducted.

Table 1. Median radiographic scores (± median absolute deviation) for CJ and TJ at days 0 and 75.

Radiographic scores	Day 0		Day 75	
	CJ Control (n=10)	TJ Treatment (n=6)	CJ Control (n=10)	TJ Treatment (n=6)
Synovial effusion (0-2)[a]	0 (±0)	0(±0)	2(±0)	1(±0)
Osteophytes (0-24) [a]	0.5 (±0.5)	1.5(±0.5)	4.5(±2.5)	4(±1)
Sclerosis (0-14)	3.5(±1.5)	1(±0)	3(±1)	2.5(±1.5)
Lysis (0-14)	0(±0)	0(±0)	0(±0)	0(±0)
Joint Space (0-2)	0(±0)	0(±0)	0(±0)	0(±0)
Total score (0-56)[a]	4(±2)	2.5(±1)	10.5(±3)	7(±1.5)

[a] Significant time effect (P < 0.01).

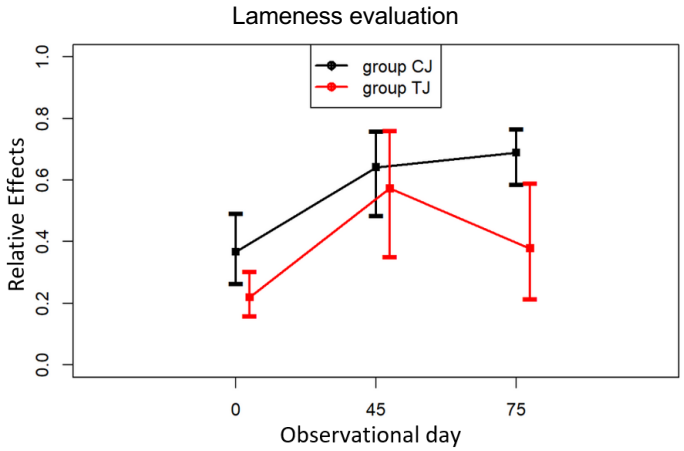

Figure 1 – Relative effects of time and treatment on lameness, detected as the total head asymmetry (vector sum, VS) above 8,5mm on the control joints (CJ) and treated joints (TJ) of the OA limbs. Significant interaction effect between treatment and time (p-value = 0.035)

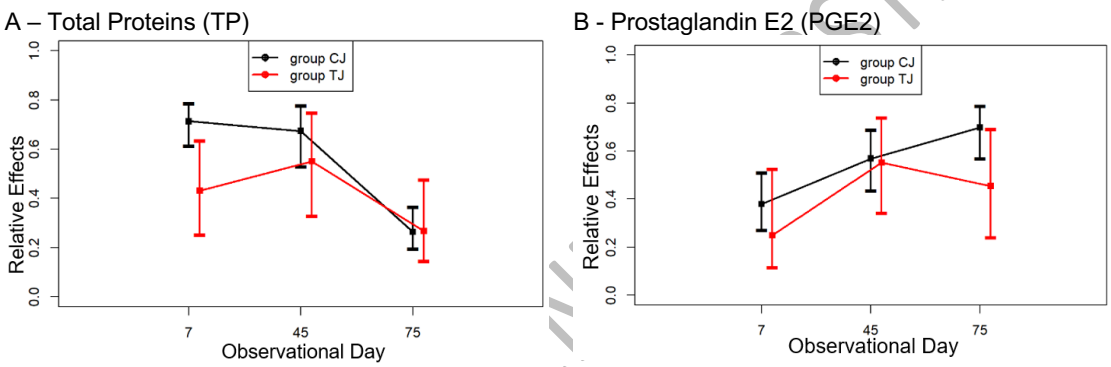

Figure 2 – A and B relative effects of time and treatment on TP and PGE2 concentration in synovial fluid of control joints (CJ) and treated joints (TJ), respectively.

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